

NUCLEOTIDE SEQUENCE OF tRNA^{Gly} FROM THE POSTERIOR SILK GLANDS OF *BOMBYX MORI*

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1. Introduction

The posterior silk glands of *Bombyx mori* exclusively produce fibroin from day 5–8 of the 5th instar. In proportion to the amino acid content of fibroin, this organ synthesizes much larger amounts of tRNAs specific for glycine, alanine and serine than those specific for the other amino acids [1,2]. Because of the characteristic synthesis and accumulation of the particular tRNA species, the silk glands should be promising as a system to study on the correlation of tRNA and mRNA syntheses. In spite of an interest to the function of the posterior silk glands, sequencing of tRNA from this organ was scarcely carried out so far. Glycine tRNA from the posterior silk glands is composed of two major species, i.e., tRNA^{Gly}₁ and tRNA^{Gly}₂ [3]*. We present here the primary structure of the tRNA^{Gly}₂.

* The two isoaccepting tRNAs^{Gly} from the posterior silk glands of *B. mori* are described [3] as tRNA^{Gly}₁ and tRNA^{Gly}₂ in the order of elution from a DEAE-Sephadex A-50 column. Here, the previous naming is changed to another one, in which they are expressed in the order of their amounts. Thus tRNA^{Gly}₁ and tRNA^{Gly}₂ in this paper respectively correspond to tRNA^{Gly}₂ and tRNA^{Gly}₁ designated [3].

Abbreviations are used according to the 1969 recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature for abbreviations and symbols for nucleic acids, polynucleotides and their constituents.

2. Materials and methods

Unfractionated tRNA was prepared from the posterior silk glands of *B. mori*, hybrid from Japanese and Chinese strains, on day 5 and 6 of the 5th instar as in [3]. Glycine tRNA₂ was isolated by chromatographic procedures on columns of DEAE-Sephadex A-50 and benzoylated DEAE-cellulose after naphthoxy-acetylation of glycyl-tRNA^{Gly}₂ as in [4]. The purity was estimated to 90% from the glycine accepting activity.

The purified tRNA^{Gly}₂ was completely digested with pancreatic RNAase and with RNAase T₁. The products were separated by chromatography on a DEAE-Sephadex A-25 column and then by rechromatography on the same column in 7 M urea in acidic conditions or an AG 1 × 2 column in acidic conditions. The fragments thus obtained were identified by further enzymatic digestion, i.e., pancreatic RNAase, RNAase T₁, RNAase T₂, RNAase U₂, RNAase P₁, snake venom phosphodiesterase, silkworm endonuclease, and bacterial alkaline phosphatase were used with slight modifications of conditions in [5,6]. In order to overlap the fragments, some larger oligonucleotides were isolated from a partial RNAase T₁ digest of the tRNA. From the results a unique sequence was deduced.

3. Results and discussion

Figure 1 shows the nucleotide sequence of *B. mori* tRNA^{Gly}₂ arranged in a clover leaf model. The chain

(cm^5U) and the methylester of cm^5U , but $^*\text{N}$ and $^{**}\text{N}$ could not be identified as any of these known nucleosides because the unknowns showed different mobility from the knowns in several solvents of thin layer chromatography. Each of the unknowns is possibly the derivatives of uridine. The nucleotide $^*\text{Np}$ was eluted together with mononucleotides from a DEAE-Sephadex A-25 column with a NaCl concentration gradient in 0.02 M Tris-HCl buffer (pH 7.5) containing 7 M urea; however, $^{**}\text{Np}$ was eluted together with dinucleotides. Accordingly, $^{**}\text{Np}$ should contain a negatively-charged group, e.g., carboxyl group, in addition to phosphate. These facts show that the purified $\text{tRNA}_{2a}^{\text{Gly}}$ is composed of $\text{tRNA}_{2a}^{\text{Gly}}$ including $^*\text{N}$ and $\text{tRNA}_{2b}^{\text{Gly}}$ including $^{**}\text{N}$. However, we cannot still separate them from each other.

From the experimental results of glycine codon-dependent glycyI-tRNA binding to ribosomes, we know that the $\text{tRNA}_2^{\text{Gly}}$ can decode GGA well and GGG weakly (unpublished data). Since both of $^*\text{N}$ and $^{**}\text{N}$ were found in the anticodon in *B. mori* $\text{tRNA}_2^{\text{Gly}}$, it remains unsolved whether or not both of the 2 anticodons, $^*\text{N}-\text{C}-\text{C}$ and $^{**}\text{N}-\text{C}-\text{C}$, can base-pair with a glycine codon GGA which is present predominantly in fibroin mRNA together with GGU [14].

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